TRADE SECRET

STUDY TITLE:	Primary Skin Irritation in Rabbits
TEST GUIDELINES:	U.S. EPA Health Effects Test Guidelines, OPPTS 870.2500 (1998) OECD Guidelines for Testing of Chemicals, Test No. 404 (2002)
AUTHOR OF ORIGINAL REPORT AND REVISION NO. 1:	
DATES STUDY COMPLETED	
ORIGINAL REPORT:	November 18, 2010
REVISION NO. 1:	November 24, 2010
PERFORMING LABORATORY:	
LABORATORY PROJECT ID:	Study Number
WORK REQUEST NUMBER:	
SERVICE CODE NUMBER:	
SPONSOR:	

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the item documented below. The item listed does not impact the validity of the study.

The test substance was characterized by the Sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

Sponsor:

Study Director: 11/24/10
Date

Sponsor: _____
Date

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	Apr 23, 2008[1]; Oct 27, 2010	Apr 23, 2008; Oct 27, 2010
In-process inspection: *Initial body weight and initiation of dosing*	Sept 8, 2010	Oct 27, 2010
Raw data audit	Oct 27, 2010	Oct 27, 2010
Draft report review	Oct 27, 2010	Oct 27, 2010
Original final report review	Nov 18, 2010	Not applicable

11/24/10
Date

[1] The protocol used for this study was reviewed by the Quality Assurance group on this date.

CERTIFICATION

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

11/24/10

Date

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STUDY INFORMATION

Substance Tested:	
Number:	
Composition:	
Purity:	See composition, above
Physical Characteristics:	
Study Initiated/Completed:	August 16, 2010 / (see report cover page)
Experimental Start/Termination:	September 8, 2010 / September 17, 2010
In-Life Initiated/Completed:	September 8, 2010 / September 17, 2010
Notebook Number(s):	

PRIMARY SKIN IRRITATION IN RABBITS

PROTOCOL NO.:

AGENCY: EPA (TSCA) and OECD

STUDY NUMBER:

SPONSOR:

SPONSOR REPRESENTATIVE:

SPONSOR STUDY MONITOR:

TEST SUBSTANCE IDENTIFICATION:

DATE RECEIVED: August 12, 2010

REFERENCE NO.:

STUDY INITIATION DATE: August 16, 2010

EXPERIMENTAL INITIATION DATE: September 8, 2010

EXPERIMENTAL COMPLETION DATE: September 17, 2010

STUDY COMPLETION DATE: November 18, 2010

1. PURPOSE

To provide information on the skin irritation potential from a single topical exposure to

2. SUMMARY

A primary skin irritation test was conducted with rabbits to determine the potential for to produce irritation after a single topical application.

At the request of the Sponsor, the study was conducted in a stepwise manner using a single patch applied initially to one rabbit for 4-hours. Five-tenths of a milliliter of the test substance was

applied to the skin of one healthy rabbit for 4- hours. The dose site was evaluated for dermal irritation by the method of Draize *et al.*[1] (see Table 4) immediately following patch removal, and at 30-60 minutes and 24, 48, and 72 hours after patch removal. Since no dermal irritation was observed in this animal, the test was completed on two additional animals, as described above.

There was no edema observed at any treated dose site during the study. Erythema (score of 1) was noted for one of the three treated dose sites immediately after patch removal and at the 30-60 minute scoring interval. This animal was free from dermal irritation by 24 hours.

In accordance with the provisions of Directive 67/548/EEC, classification by the dermal route is not required.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, classification is not required.

3. MATERIALS

A. Test Substance

The test substance, identified as was received on August 12, 2010 and was further identified with Reference Number The test substance was stored at room temperature. The sample was applied as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the test substance was provided by the Sponsor:

Physical description:

pH:

Solubility: Not provided

Stability: The test substance was expected to be stable for the duration of testing

Expiration Date: March 1, 2011

The test substance will be retained for at least 3 months following submission of the final report, unless otherwise specified by the Sponsor. After this time period all remaining test substance will be properly disposed. Records of sample disposition are maintained by .

B. Animals

3.B.1 Number of Animals: 3

3.B.2 Sex: Male

3.B.3 Species/Strain: Rabbit/New Zealand albino.

3.B.4 Age: Young adult.

[1] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

3.B.5 Source: Received from Robinson Services, Inc. Clemmons, NC on September 1 and 8, 2010.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 1996). Enrichment (e.g. toy) was placed in each cage. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 19-22 °C and 45-69%, respectively.

4.A.3 Photoperiod: 12 hour light/dark cycle

4.A.4 Acclimation Period: 6 or 7 days

4.A.5 Food: Pelleted Purina Rabbit Chow #5326

4.A.6 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.

4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at

B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: A number was allocated to each rabbit on receipt and a stainless steel ear tag bearing this number was attached to the animal. This number, together with a sequential animal number assigned to study constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

Approximately 24 hours prior to application, a group of animals was prepared by clipping the dorsal area and the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy naive animals (not previously tested) without pre-existing skin irritation were selected for test.

Initially, only one rabbit was placed on test. Since there was no dermal irritation observed in this animal, the test was completed with two additional rabbits.

B. Application of Test Substance

Five-tenths of a milliliter of the test substance was applied to one 6-cm^2 intact dose site on each animal and covered with a 1-inch x 1-inch, 4-ply gauze pad. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the

pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages. No other substances were tested on these rabbits.

After 4 hours of exposure to the test substance, the pads and collars were removed and the test sites were gently cleansed of any residual test substance.

C. Evaluation of Test Sites/Classification Schemes

Individual dose sites were scored according to the Draize scoring system[1] (see Table 4) immediately following patch removal and at approximately 30-60 minutes and 24, 48, and 72 hours after patch removal.

The observed dermal effects were classified as follows:

European Economic Community (EEC)

Mean values for each lesion (erythema and edema) were calculated for each animal separately from numerical scores obtained at the 24-, 48-, and 72-hour observations. The results were interpreted according to 67/548/EEC relative to the general classification and labeling requirements for dangerous substances.

Corrosive

The test substance will be considered to be "CORROSIVE" and will require the symbol "C" and the indication of danger "CORROSIVE" if it produces full thickness destruction of the skin tissue on at least 1 animal during the skin irritation test.

Risk phrases will be assigned in accordance with the following criteria.

R35 "CAUSES SEVERE BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 3 minutes exposure or if this result can be predicted.

R34 "CAUSES BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 4 hours exposure or if this can be predicted.

Irritant

The test substance will be classified as "IRRITANT" and will require the symbol "Xi" and the indication of danger "IRRITANT" in accordance with the criteria given below.

In addition, the following risk (R) phrase will be assigned to substances, if appropriate, according to the criteria indicated below:

[1] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

R38 "IRRITATING TO SKIN"

If, when applied to healthy intact animal skin for up to 4 hours, significant inflammation is caused and is present 24 hours or more after the end of the exposure period. Inflammation is significant if the mean values of the scores for either erythema and eschar formation or edema formation corresponds to one or more of the following mean values calculated for each animal separately and has been observed in 2 or more animals:

- Erythema and eschar formation 2.0 or more
- Edema 2.0 or more

All scores at each of the reading times (24, 48, and 72 hours) for an effect should be used in calculating the respective mean values.

An R38 "IRRITATING TO SKIN" phrase should also be assigned if:

The inflammation persists in at least 2 animals at the end of the observation time. Particular effects such as hyperplasia, scaling, discoloration, fissures, scabs, and alopecia should be taken into account.

Globally Harmonized System (GHS)

Mean values for each lesion (erythema and edema) were calculated for each animal separately from numerical scores obtained at the 24-, 48-, and 72-hour observations. The results were interpreted according to United Nations Economic Commission for Europe, Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Part 3 - Health Hazards.

Skin Corrosive Category and Subcategories			
Corrosive (Category 1)	***Corrosive subcategories***	***Corrosive in ≥ 1 of 3 animals***	
		Exposure	***Observation***
corrosive	1A	≤ 3 minutes	≤ 1 hour
	1B	> 3 minutes ≤ 1 hour	≤14 days
	1C	> 1 hour ≤ 4 hours	≤ 14 days

Skin Irritation Categories	
Categories	***Criteria***
Irritant (Category 2)	(1) Mean value of ≥ 2.3 < 4.0 for erythema/eschar or for edema in at least 2 of 3 tested animals from scores at 24, 48, and 72 hours after patch removal, or if reactions are delayed, from scores on 3 consecutive days after the onset of skin reactions; or (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or (3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.
Mild Irritant (Category 3)	Mean value of ≥ 1.5 < 2.3 for erythema/eschar or for edema from scores in at least 2 of 3 tested animals from scores at 24, 48, and 72 hours or, if reactions are delayed, from scores on 3 consecutive days after the onset of skin reactions (when not included in the irritant category above).

(Reference: United Nations Economic Commission for Europe, Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Part 3 – Health Hazards).

D. Body Weights

Individual body weights of the animals were recorded prior to test substance application (initial) and again on the last day of individual scoring.

E. Clinical Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

6. ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals *(Natl. Res. Council, 1996)*. All studies conducted for adhere to the following principles:

- The sponsor ensures that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.
- policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- Animals were provided with species-appropriate environmental enrichment.

is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

7. STUDY CONDUCT

This study was conducted at

The study director for this study was

The primary scientist for this study was with contributions by .

8. TEST GUIDELINES

The procedures as described in the protocol are based on the following testing guidelines:

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2500 (1998)

OECD Guidelines for Testing of Chemicals, Test No. 404 (2002)

9. QUALITY ASSURANCE

The final report was audited for agreement with th e raw data records and for compliance with the protocol and Standard Operating Pr ocedures. Dates of inspections and audits performed during the study, and the dates of repor ting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

10. DEVIATIONS FROM THE FINAL PROTOCOL

None.

11. FINAL REPORT AND RECORDS RETENTION

A copy of th e signed repo rt, copies of all raw data generated at and the orig inal signed protocol and amendments (if any), will be maintained in the archives. will maintain these records for a period of at least five years.

Laboratory-specific or site-specific raw data, su ch as personnel files and equipment record s will be retained by the facility where the work was done.

Specimens (i f applicable), raw data, an d a copy of the protocol and am endments (if any), and the final report will be retained at

12. RESULTS

Individual body weights are presented i n Table 1. Individual skin irritation scores are presented in Table 2. A su mmary of mean scores calculated in accordance with EEC/G HS is pres ented in Table 3. The Draize Primary Skin Irritation Scoring System is presented in Table 4.

All animals a ppeared active and healthy and gained body weight during the st udy. Apart f rom the dermal irritation noted below, there were no other clinical signs of toxicity.

There was no ede ma obser ved at any treated dose site during the study. Erythema (score of 1) was noted for one of the three treated dose sites immediately after patch removal and at the 30-60 minute scoring interval. This animal was free from dermal irritation by 24 hours.

13. CONCLUSION

In accordance with the provisions of D irective 67/548/EEC, classification by the dermal route i s not required.

According to the Globally Harmonized System (GHS) of classification and labeling of chem icals and under the conditions of this study, classification is not required.

TABLE 1: INDIVIDUAL BODY WEIGHTS

Animal No.	Sex	Body Weight (g)	
		Initial	Terminal
3501	M	2241	2312
3502	M	2018	2158
3503	M	1995	2108

TABLE 2: INDIVIDUAL SKIN IRRITATION SCORES

ERYTHEMA/EDEMA

Animal No.	Sex	Time After Patch Removal				
		Immediately after patch removal	30-60 mins	24 hrs	48 hrs	72 hrs
3501	M	0/0	0/0	0/0	0/0	0/0
3502	M	0/0	0/0	0/0	0/0	0/0
3503	M	1/0	1/0	0/0	0/0	0/0

TABLE 3: SUMMARY OF MEAN SCORES FOR DERMAL RESPONSES

RABBIT NUMBER	ERYTHEMA[a]	EDEMA[a]
3501	0.0	0.0
3502	0.0	0.0
3503	0.0	0.0

a Calculated from the 24-, 48-, and 72-hour dermal responses (EEC/GHS).

TABLE 4: PRIMARY SKIN IRRITATION SCORING SYSTEM

Evaluation of Skin Reactions	Value
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

APPENDIX A: REVISION 1 EXPLANATION

The report was revised as follows:

Page(s)	
6	The test substance tested and 1st ingredient under composition on the Study Information page was changed.
18	Appendix A: Revision 1 added.

The following pages were revised to reflect these changes.

Page	
1	Title Page
5	Table of Contents